Characterization of Multidrug-resistant and Virulent *Klebsiella Pneumoniae* Strains Belonging to the High-risk CG258 Isolated from Inpatients in Northeastern Brazil

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Characterization of multidrug-resistant and virulent *Klebsiella pneumoniae* strains belonging to the high-risk CG258 isolated from inpatients in northeastern Brazil

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Abstract

Multidrug-resistant (MDR) and hypervirulent *Klebsiella pneumoniae* (hvKp) clones have become a major threat to global public health. The CG258 is considered a high-risk CG and the *K. pneumoniae* strains belonging to it are known to be often multi-resistant and to spread mainly in the hospital environment. This study aimed to characterize the antimicrobial resistance profile, virulence factors, and the clonal relationships among 13 *K. pneumoniae* strains belonging to CG258 from patients admitted to a tertiary hospital in Teresina, in the state of Piauí, northeastern Brazil. Ten strains were classified as MDR and three as extensively drug-resistant (XDR). Three different β -lactamase-encoding genes (*bla*_{KPC}, *bla*_{OXA-1-like}, and *bla*_{CTX-M-Gp1}) and six virulence genes (*fimH*, *ycfM*, *mrkD*, *entB*, *ybtS*, and *kfu*) were detected. Moreover, two hypermucoviscous *K. pneumoniae* strains and one capsular K-type 2 were found. Multilocus sequence typing analysis revealed 10 different sequence types (STs) (ST14, ST17, ST20, ST29, ST45, ST101, ST268, ST1800, ST3995, and ST3996) belonging to CG258, being two (ST3995 and ST3996) described for the first time in this study.

Keywords: *Klebsiella pneumoniae*, KPC, MDR, Hypervirulent, Hypermucoviscous, CG258.

Introduction

Klebsiella pneumoniae is a Gram-negative bacillus, usually encapsulated, nonmotile bacterium and belongs to the *Enterobacteriaceae* family. It is an opportunistic pathogen that can cause several infections and is among the most common nosocomial pathogens worldwide (Wyres et al. 2020). *K. pneumoniae* strains can contain a wide range of virulence and antimicrobial resistance factors (Ashurst et al. 2020). Several virulence factors further increase the severity of infections by *K. pneumoniae*, such as siderophores that are systems of chelating molecules that can competitively eliminate iron from host proteins and other sources; capsules, including their overproduction characterized by the hypermucoviscous (HM) phenotype; among others (Paczosa et al. 2016).

The presence of clinically relevant antimicrobial resistance genes (ARGs), including extended-spectrum β -lactamases (ESBLs) and carbapenemases-encoding genes can limit therapeutic options, making it difficult to treat infections in affected patients (Piperaki et al. 2017). Multidrug-resistant (MDR), and hypervirulent *K. pneumoniae* (hvKp) clones have become major global health problems (Wyres et al. 2020).

This study aimed to determine the pathogenic potential, antimicrobial resistance profile, and the clonal relationships among *K. pneumoniae* strains belonging to CG258 from patients admitted to hospitals in northeastern Brazil.

Material and methods

Bacterial strains

This study included 13 *K. pneumoniae* strains isolated from different patients admitted to a tertiary hospital in Teresina, a Brazilian municipality located in the northeast region, being capital of the state of Piauí, with an estimated population of 868,075

inhabitants. The strains were randomly isolated between August to September 2013 from several sources (catheter tip, hemoculture, purulent secretion, tracheal secretion, and urine) (Table 1). The strains were identified by the VITEK 2 system (bioMérieux, France) and confirmed by the 16S rRNA sequencing, after amplification and sequencing with the primers fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rP2 (5'-ACGGCTACCTTGTTACGACTT-3'), according to Weisburg et al. (1991). The strains were maintained at -80 °C in 15 % glycerol for subsequent experiments.

Hypermucoviscosity test

The HM phenotype was investigated using the string test according to Wiskur et al. (2008). The strains were inoculated on Mueller-Hinton agar (Oxoid Ltd., United Kingdom) and incubated for 18 h at 37 °C. After bacterial growth, using a bacteriological loop, an isolated colony was touched and raised vertically. The HM phenotype was considered positive when there was the formation of a viscous filament ≥ 5 mm.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was realized by the disk diffusion method on Mueller-Hinton agar (Oxoid Ltd., United Kingdom) following the recommendations of the Clinical Laboratory Standards Institute (CLSI, 2020) and the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST, 2020). For this approach, 40 different antimicrobial disks (Oxoid Ltd., United, Kingdom) recommended for *Enterobacteriales* were used. The susceptibility for colistin (COL) was determined by the broth microdilution method (CLSI, 2020). Each strain was considered susceptible or non-susceptible (either intermediate or resistant) to each antimicrobial tested. The antimicrobials tested are shown in Figure 1.

The strains *Escherichia coli* ATCC® 25922 and *Pseudomonas aeruginosa* ATCC® 27853 were used as controls in these experiments. Based on the susceptibility profile, the *K. pneumoniae* strains were classified as MDR, extensively drug-resistant (XDR), or pandrug-resistant (PDR) according to Magiorakos et al. (2012).

Detection of virulence genes and ARGs by Polymerase Chain Reaction (PCR)

The strains were subjected to conventional PCR assays to detect K1 (*magA*, K1 capsule-specific *wzy* gene) and K2 capsular serotypes and nine gene encoding virulence factors: *fimH* and *mrkD* (adhesins), *ycfM* (lipopolysaccharide), *entB*, *iutA*, *ybtS*, *kfu* (iron acquisition systems), *allS* (allantoin metabolism) and *rmpA* (capsular serotype and HM regulator phenotype) (Conpain et al. 2014; Fang et al. 2004; Yu et al. 2007). Moreover, 17 ARGs were investigated, including 16 β -lactamase-encoding genes (*bla*_{GES}, *bla*_{IMP}, *bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-1-like}, *bla*_{OXA-48-like}, *bla*_{VIM}, *bla*_{SPM}, *bla*_{GIM}, *bla*_{CTX-M-Gp1}, *bla*_{CTX-M-Gp2}, *bla*_{CTX-M-Gp8}, *bla*_{CTX-M-Gp9}, *bla*_{CMY-2}, *bla*_{VEB}, and *bla*_{BEL}) and the COL resistance gene *mcr-1* (Clímado et al. 2013; Dallenne et al. 2010; Ellington et al. 2007; Liu et al. 2016; Peirano et al. 2011; Pitout et al. 2005; Poirel et al. 2010).

One amplicon from each gene found was randomly selected to confirm its identity by sequencing using an automated sequencer (ABI 3500xL Genetic Analyzer; Applied Biosystems, USA). The obtained sequences were compared with those available in GenBank using the BLAST algorithm (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The *ycfM* gene, although confirmed by sequencing, was not deposited due to its small size amplicon (< 200 bp). In addition, all *wzi* genes were directly analyzed and deposited, when necessary, in the *K. pneumoniae* MLST database (<https://bigsd.b.pasteur.fr/klebsiella/klebsiella.html>).

Determination of capsular type

The capsular type (K-type) of the strains was determined by amplification and sequencing of the *wzi* gene as described by Brisse et al. (2013). The *wzi* gene was amplified by PCR and the amplicons were sequenced using the automated sequencer (ABI 3500xL Genetic Analyzer; Applied Biosystems, USA). Subsequently, the obtained sequences were submitted to the *K. pneumoniae* MLST database (<https://bigsd.b.pasteur.fr/klebsiella/klebsiella.html>) to determine the alleles corresponding to each K-type and K-locus of the strains.

Multilocus Sequence Typing (MLST)

The strains were evaluated by the MLST technique using protocol 2 of the *K. pneumoniae* MLST database (<http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html>). To show the clonal relationships among sequence types (STs) and clonal groups (CGs), goeBURST 1.2.1 version (<http://www.phyloviz.net/goeburst>) was used.

Results and discussion

Antimicrobial resistance profiles

The majority of strains were non-susceptible to nitrofurantoin (NIT), ciprofloxacin (CIP), and trimethoprim-sulfamethoxazole (SUT) (n = 13, 92.8 %), the other members of the class of folate pathway antagonists also demonstrated high percentages, such as sulphonamide (SUL) and trimethoprim (TRI) (both 85.7 %). Similar results were obtained for the tetracyclines class, such as doxycycline (DOX), tetracycline (TET) (both 85.7 %) and minocycline (MIN) (78.6 %). All strains were susceptible to cefotetan (CTT). Almost all were susceptible to COL since the minimal inhibitory

concentration (MIC) found was < 1 mg/L, except for the KpPi151 strain that showed resistance to COL (MIC 32 mg/L). However, the *mcr-1* gene was not found in this strain, indicating that resistance to COL is due to other genes or mechanisms not investigated in this study, as described by El-Sayed et al. (2020). Figure 1 shows the percentage of non-susceptibility of each antimicrobial.

A large and worrying level of multidrug resistance strains was found, being ten strains classified as MDR and three strains (KpPi150, KpPi156 and KpPi159) as XDR (Table 1). None of the strains were classified as PDR. Among these strains, seven (KpPi145, KpPi146, KpPi147, KpPi149, KpPi151, KpPi155, and KpPi156) did not present any of the investigated ARGs, suggesting that other genes or mechanisms not researched in this study may be involved, such as efflux pumps, gene target modifiers, and changes in antimicrobial targets by mutations (Blair et al. 2015). The ideal to fully characterize the ARGs among the strains would be to characterize their resistomes by whole genome sequencing, which was not the aim of the study. Many *K. pneumoniae* strains with multiple antimicrobial resistance have been reported around Brazil. MDR and XDR *K. pneumoniae* strains have been found in the north (Ferreira et al. 2019), northeast (Aires et al. 2017), southeast (Braun et al. 2018), and south (Nava et al. 2019). In a study by Gonçalves et al. (2017), a total of 26 strains of *K. pneumoniae*, including 14 MDR, seven XDR and three PDR strains were found in a university hospital in southern Brazil, corroborating with our results.

Presence of ARGs and the relationship with antimicrobial resistance profiles

Three different ARGs were detected among the strains, including *bla*_{OXA-1-like}, *bla*_{CTX-M-Gp1} and *bla*_{KPC} (GenBank accession numbers MT330307, MT330309 and MT330311). The *bla*_{OXA-1-like} was detected in four strains - KpPi148, KpPi152, KpPi153

and KpPi159 (Table 1). According to Sugumar et al. (2014), the production of OXA-1 and other β -lactamases results in resistance to ampicillin, piperacillin, ticarcillin and cephalosporins; in fact, this profile was observed in our results. Four strains (KpPi148, KpPi152, KpPi153, and KpPi159) demonstrated resistance to APS, PIT, TAC, CAZ, CFC, CFM, CFZ, CPM, CRO, CRX, CTL, CTX and CFO, and the only exceptions were CFO for which two strains (KpPi148 and KpPi152) were susceptible and for CTT which all strains were susceptible. All tested antibiotics are shown with their abbreviations in Figure 1.

The KpPi152 strain also has *bla*_{CTX-M-Gp1}, being the unique strain to present this gene. Chagas et al. (2011) studied the diversity of genotypes in 38 CTX-M-producing *K. pneumoniae* from different Brazilian hospitals located in three southeastern cities and suggested that the intensive use of broad-spectrum cephalosporins could be responsible for the amount of *bla*_{CTX-M} found. The KpPi152 strain presented resistance to cephalosporins CAZ, CFC, CFM, CFZ, CPM, CRO, CRX, CTL and CTX.

Three strains (KpPi150, KpPi153 and KpPi157) harbored the *bla*_{KPC} gene (Table 1). KPC-producing bacteria are closely related to infections associated with a high-level of morbidity and mortality because carbapenem antimicrobials are generally not effective against these bacteria (Arnold et al. 2011). The KpPi153 strain presented resistance to all tested carbapenems (DOR, ERT, IPM, and MPM) and KpPi150 strain to IPM and MPM. Interestingly, although the KpPi157 strain presented the *bla*_{KPC} gene, it was susceptible to carbapenems. On the other hand, as described by Marschall et al. (2009) and Villegas et al. (2006), the presence of the *bla*_{KPC} gene may not always result in resistance *in vitro* to carbapenems, resulting in a possible failure to detect this phenotype during routine workup. Therefore, an accurate investigation to detect KPC and the real resistance to

carbapenems is crucial to establish control of their silent dissemination in strains that remain susceptible *in vitro* (Marschall et al. 2009; Villegas et al. 2006).

Occurrence of virulence genes and HM phenotype

Several virulence genes were found among the strains. All strains presented the genes *fimH* and *ycfM*, followed by *mrkD* (92.3 %), *entB* (69.2 %), *ybtS* (53.8 %), and *kfu* (38.5 %) (GenBank accession numbers MT330315, MT330318, MT330321, MT330323, MT330325, and MT330326) (Table 1). The high prevalence of *fimH*, *mrkD* and *entB* genes found in this study was already expected, since several studies have shown a high correlation of these genes in *K. pneumoniae* strains isolated from different sources and countries (Azevedo et al. 2019; Ferreira et al. 2019; Gonçalves et al. 2017). Similar results were obtained by Kuş et al. (2017), who demonstrated that the four virulence genes most detected in *K. pneumoniae* strains of nosocomial infections were *entB* (96.2 %), *ycfM* (86.8 %), *mrkD* (83 %), and *fimH* (64.2 %). The strains KpPi149 and KpPi150 presented HM phenotype in addition to being MDR and XDR, respectively. The *rpmA* and *magA* genes are associated with this phenotype and some studies have shown that *magA* is associated to the K1 capsule-specific *wzy* gene (Catalán-Nájera et al. 2017). However, none of these genes were detected in both strains. This characteristic has already been reported in some *Klebsiella* spp., including *K. pneumoniae* (Lee et al. 2010; Yu et al. 2006; Mohammed and Flayyih 2018). Recently, an HM *Klebsiella variicola* subsp. *variicola* without the genes *rpmA* and *magA* was described causing primary endodontic infection and none of these genes were detected (Nakamura-Silva et al. 2020). These studies corroborate our results indicating that other genes may be involved in the HM phenotype.

Molecular typing of *K. pneumoniae* strains

MLST analysis showed 10 different STs (ST14, ST17, ST20, ST29, ST45, ST101, ST268, ST1800, ST3995, and ST3996) (Table 1). Two STs (ST3995 and ST3996) were described for the first time in this study, since the strains presented new alleles for *tonB* gene (allele 573 for ST3995 and allele 574 for ST3996). All STs were found to belong to CG258 (Figure 2), which is considered a high-risk CG and is well known for spreading ARGs, mainly *bla_{KPC}* and virulence genes worldwide (Pitout et al., 2015). CG258 and ST11 have been described as widespread in Brazil (Gonçalves et al. 2017), including outpatient infections (Azevedo et al. 2019).

The new ST3996 was found in an XDR strain (KpPi156) which is a single-locus variant (SLV) of ST11 (Figure 2). Two MDR strains (KpPi146 and KpPi153) presented ST101, which is considered an emerging clone that has been identified worldwide and has the potential to become a persistent threat to global public health (Roe et al. 2019). Moreover, the Kpi153 strain presented the *bla_{KPC}* gene (Table 1). These characteristics were also found in southern Brazil and Italy (Gonçalves et al. 2017; Roe et al. 2019). Another new ST found in this study was ST3995 that was described in the strains Kpi152 and Kpi159, which is also an SLV of ST101. The appearance of strains with the same ST in different patients and sources suggests the spread of these STs, a characteristic observed in ST3995, ST101 and ST17 (Table 1).

The Kpi145 strain presented ST268/KL20, which corroborates with several other studies that demonstrate an association between ST268 and K20 in *K. pneumoniae* strains (Table 1) (Liu et al. 2014; Lin et al. 2015; Yan et al. 2015; Yan et al. 2016; Zhang et al. 2016, Guo et al. 2017; Chen et al. 2017). The KpPi148 strain (ST14) harbors the *bla_{OXA-1-like}* gene and other β -lactamase-encoding genes that have already been described in this ST, such as *K. pneumoniae* strains harboring *bla_{CTX-M-15}* causing neonatal sepsis in

Tanzania (Mshana et al. 2013), and *K. pneumoniae* co-producing *bla*_{OXA-48} and *bla*_{NDM} carbapenemases in Dubai (Moubareck et al. 2018). Furthermore, the KpPi148 strain presented the capsular serotype K2 which is frequently associated with invasive infections when found in hv clones (Wyres et al. 2019); however, ST14 is a non-hv clone and *K. pneumoniae* ST14/K2 strains have been found in several other studies (Brisse, et al. 2009; Harada et al. 2018; Musicha et al. 2019).

The KpPi147 presented ST45, *wzi* allele 101, and associated K-type 24 (Table 1). These same characteristics were found in an outbreak caused by MCR-1-producing *K. pneumoniae* strains (24 strains of ST45 and one of ST1112) isolated from patients in a hospital in Porto, Portugal (Mendes et al. 2018). Moreover, in a study of ESBL-producing *Enterobacteriaceae* causing sepsis in neonates at a tertiary hospital in Tanzania, 18 out of 38 *K. pneumoniae* strains showed ST45, being the most common ST among the strains; however, unlike KpPi147, all *K. pneumoniae* ST45 strains in the African study presented the *bla*_{CTX-M-15} gene (Marando et al. 2018). ST20 has already been reported causing outbreaks in neonatal wards. Between 2012 and 2013, Jin et al. (2015) described two outbreaks in China involving MDR *K. pneumoniae* ST20 and ST17 harboring *bla*_{NDM} and *bla*_{KPC}. In comparison, our strain KpPi155 (ST20) did not show any of these genes. In a recent review of the genomic population of *K. pneumoniae*, Wyres et al. (2020) described eight MDR global problem *K. pneumoniae* clones, including ST101 and ST20, which were found in our strains (Table 1).

The strains KpPi149 and KpPi150 presented ST17, both have *wzi* allele 141, similar virulence genes, and HM phenotype, but only Kpi150 presented the *bla*_{KPC} gene and an XDR profile (Table 1). ST17 has also been shown to carry other ARGs in southern Brazil, such as *bla*_{OXA-370} and *bla*_{CTX-M-8} (Aires et al., 2016). In addition, ST17 was also found in a CTX-M-15-producing *K. pneumoniae* strain in Norway, which caused an

outbreak in a neonatal intensive care unit and subsequent intestinal colonization of affected children for up to two years (Löhr et al. 2015). Some STs found in this study were described as causing neonatal outbreaks in other countries (Mshana et al. 2013; Marando et al. 2018; Jin et al. 2015; Löhr et al. 2015). However in the present study, none of the strains were isolated from neonates, with the average age of the patients being 57 years old and the youngest a child of 3 years old (KpPi159) (Table 1).

In the present study, the strain KpPi151 presented ST29 with *wzi* allele 85 and K-type 30. Moura et al. (2017) analyzed the genome sequence of an ST29 HM/hv MDR CTX-M-15 *K. pneumoniae* strain isolated from human infection in southeastern Brazil; however, they found the *wzi* allele 19 and K-type 19 for this strain. To the best of our knowledge, there are no reports of the ST1800 of *K. pneumoniae* ST1800 (KpPi157) in the literature, where only two strains are deposited in the *K. pneumoniae* MLST database (<https://bigsd.b.pasteur.fr/klebsiella/klebsiella.html>), however without any information about them. The *wzi* sequences submitted to the MLST database did not find alleles corresponding to K-type for the strains KpPi149, KpPi150, KpPi153, and KpPi155 (Table 1).

Conclusions

The results presented in this study raise worrying data related to multidrug resistance and virulence in *K. pneumoniae* strains isolated from inpatients in the northeast of Brazil. In addition, all STs found, including two new STs, belonged to CG258, an international high-risk CG. Therefore, this study contributes to epidemiological surveillance studies in the monitoring of MDR, XDR, and virulent clones pertaining to CG258 worldwide.

Declarations

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Ethical approval Ethical approval was received from the School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (Ribeirão Preto, SP, Brazil) [approval no. CEP/FCFRP 362; CAEE 36031914.9.0000.5403].

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Conflict of interest The authors declare that they have no competing interests.

Author contributions André Pitondo-Silva designed this study, contributed to the analysis and with intellectual experience. Rafael Nakamura-Silva carried out all experiments and analyzes of this study and wrote the manuscript. Mariana Oliveira-Silva contributed to DNA extraction, antimicrobial susceptibility testing, hypermucoviscosity test and PCRs. Carlos E. S. Miranda provided the bacterial collection. João Pedro R.

Furlan and Eliana G. Stehling contributed to the analysis of results. All authors reviewed and approved the final manuscript.

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Caption of tables and figures

Table 1. General data related to 13 *K. pneumoniae* strains used in this study.

Figure 1: Frequency of non-susceptibility found among the 13 strains of *K. pneumoniae* studied. Each color represents a class of antimicrobials. Antimicrobials tested: amoxicillin-clavulanate (AMC), amikacin (AMI), ampicillin-sulbactam (APS), aztreonam (ATM), ceftazidime (CAZ), cefaclor (CFC), cefixime (CFM), ceftiofur (CFO), cefazolin (CFZ), ciprofloxacin (CIP), chloramphenicol (CLO), colistin (COL), cefepime (CPM), ceftriaxone (CRO), cefuroxime (CRX), ceftaroline (CTL), cefotetan (CTT), cefotaxime (CTX), doripenem (DOR), doxycycline (DOX), ertapenem (ERT), streptomycin (EST), fosfomicin (FOS), gentamicin (GEN), imipenem (IPM), levofloxacin (LEV), lomefloxacin (LMX), minocycline (MIN), meropenem (MPM), nalidixic acid (NAL), netilmicin (NET), nitrofurantoin (NIT), norfloxacin (NOR), ofloxacin (OFX), piperacillin-tazobactam (PIT), sulphonamide (SUL), trimethoprim-sulfamethoxazole (SUT), ticarcillin-clavulanate (TAC), tetracycline (TET), tobramycin (TOB), trimethoprim (TRI).

Figure 2: A goeBurst diagram representing the clonal relationship among STs of *K. pneumoniae* strains indicating clonal group 258 (CG258) (blue) and sequence types (STs) found (red).

Figure 2

Table 1. General data related to 13 *K. pneumoniae* strains used in this study

Strain	Age (years)	Source	ARP ¹	ARGs (<i>bla</i>) ²	Virulence genes	HM ³	<i>wzi</i> allele	K-type ⁴	K-locus	ST
KpPi145	71	Tracheal secretion	MDR	None	<i>entB, mrkD, fimH, ycfM</i>	-	95	K20	KL20	268
KpPi146	65	Hemoculture	MDR	None	<i>kfu, entB, mrkD, ybtS, fimH, ycfM</i>	-	137	K17	KL17	101
KpPi147	59	Tracheal secretion	MDR	None	<i>entB, mrkD, ybtS, fimH, ycfM</i>	-	101	K24	KL24	45
KpPi148	62	Hemoculture	MDR	OXA-1	<i>kfu, entB, mrkD, K2, fimH, ycfM</i>	-	2	K2	KL2	14
KpPi149	82	Hemoculture	MDR	None	<i>entB, mrkD, ybtS, fimH, ycfM</i>	+	141	ND	KL25	17
KpPi150	77	Urine	XDR	KPC	<i>mrkD, ybtS, fimH, ycfM</i>	+	141	ND	KL25	17
KpPi151	19	Catheter tip	MDR	None	<i>entB, mrkD, fimH, ycfM</i>	-	85	K30	KL30	29
KpPi152	25	Hemoculture	MDR	OXA-1, CTX-M-1	<i>kfu, ybtS, fimH, ycfM</i>	-	137	K17	KL17	3995
KpPi153	39	Tracheal secretion	MDR	OXA-1, KPC	<i>kfu, mrkD, ybtS, fimH, ycfM</i>	-	356	ND	KL117	101
KpPi155	81	Hemoculture	MDR	None	<i>entB, mrkD, fimH, ycfM</i>	-	160	ND	KL39	20
KpPi156	69	Tracheal secretion	XDR	None	<i>entB, mrkD, fimH, ycfM</i>	-	100	K10	KL10	3996
KpPi157	79	Urine	MDR	KPC	<i>entB, mrkD, fimH, ycfM</i>	-	25	K25	KL25	1800
KpPi159	3	Purulent secretion	XDR	OXA-1	<i>kfu, mrkD, ybtS, fimH, ycfM</i>	-	137	K17	KL17	3995

¹ ARP, antimicrobial resistance profile; MDR, multidrug-resistant; XDR, extensively drug-resistant.

² ARGs, antimicrobial resistance genes.

³ HM, hypermucoviscous phenotype.

⁴ ND, not determined.

Figures

Figure 1

Frequency of non-susceptibility found among the 13 strains of *K. pneumoniae* studied. Each color represents a class of antimicrobials. Antimicrobials tested: amoxicillin-clavulanate (AMC), amikacin (AMI), ampicillin-sulbactam (APS), aztreonam (ATM), ceftazidime (CAZ), cefaclor (CFC), cefixime (CFM), ceftazidime (CFO), cefazolin (CFZ), ciprofloxacin (CIP), chloramphenicol (CLO), colistin (COL), cefepime (CPM), ceftriaxone (CRO), cefuroxime (CRX), ceftaroline (CTL), cefotetan (CTT), cefotaxime (CTX), doripenem (DOR), doxycycline (DOX), ertapenem (ERT), streptomycin (EST), fosfomicin (FOS), gentamicin (GEN), imipenem (IPM), levofloxacin (LEV), lomefloxacin (LMX), minocycline (MIN), meropenem (MPM), nalidixic acid (NAL), netilmicin (NET), nitrofurantoin (NIT), norfloxacin (NOR), ofloxacin (OFX), piperacillin-tazobactam (PIT), sulphonamide (SUL), trimethoprim-sulfamethoxazole (SUT), ticarcillin-clavulanate (TAC), tetracycline (TET), tobramycin (TOB), trimethoprim (TRI)

Figure 2

A goeBurst diagram representing the clonal relationship among STs of *K. pneumoniae* strains indicating clonal group 258 (CG258) (blue) and sequence types (STs) found (red)